The effect of a combination of decapitation treatments, zeatin and benzyladenine on the initiation and emergence of lateral roots in *Pisum sativum*

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Seedlings of *Pisum sativum* cv. Greenfeast were used to determine the influence of the root tip, cotyledons and root-applied cytokinins on lateral root initiation and development. Root tip decapitation resulted in a rapid increase in the number of lateral root primordia initiated. The number of emerged lateral roots increased by 30% over the control. Removal of the shoot tip and cotyledons alone, or in combination with the root tip, reduced lateral root emergence and inhibited the number of lateral root primordia initiated. Zeatin and benzyladenine at 10^{-6} M and above inhibited both initiation and emergence of lateral roots. The effect of cytokinin treatments was more pronounced on emergence of lateral roots than on the initiation phase. High concentrations of cytokinin maintained apical dominance of the root even in root tip-decapitated seedlings. It appears as if cytokinins of root tip origin inhibit lateral root formation in the 10–15-mm apical section of the primary root. Both cotyledons and the root tip apparently control the initiation and emergence of the lateral roots.

Pisum sativum cv. Greenfeast-saailinge is gebruik om die effek van die wortelpunt, saadlobbe en wortel-toegediende sitokiniene op sywortelinisiasie en ontwikkeling te bestudeer. Wortelpuntverwydering het tot 'n vinnige toename in die aantal wortelprimordia gelei. Die aantal wortels wat verleng het, het met 30% toegeneem ten opsigte van die kontrole. Verwydering van die stingelpunt en saadlobbe alleen, of in kombinasie met die wortelpunt, het sywortelontwikkeling verminder en die aantal sywortelprimordia wat gevorm is geïnhibeer. Zeatin en bensieladenien teen 10^{-6} M en hoër het die inisiasie en verlenging van sywortels geïnhibeer. Die effek van sitokinien-behandelings was meer opmerklik op verlenging van die sywortels as op inisiasie. Hoë konsentrasies sitokinien het apikale oorheersing van die wortelpunt in stand gehou, selfs waar die wortelpunt verwyder is. Dit skyn asof sitokiniene van wortelpuntoorsprong sywortel-vorming in die 10–15-mm apikale gedeelte van die primêre wortel inhibeer. Beide saadlobbe en die wortelpunt beheer skynbaar die inisiasie en verlenging van die sywortels.

Keywords: Cytokinins, lateral root formation, root apical dominance.

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Introduction

The initiation and emergence of lateral root primordia are regulated temporally, spatially and numerically by many factors. After germination, the cotyledons and the primary root are responsible for establishing the plant successfully. The formation of lateral roots in acropetal sequence, at a distance behind the root tip, would suggest that it is under genetic, hormonal or nutritional control of the root tip and/or other parts of the seedling (Torrey 1956; Keller & Lamb 1989).

Cytokinin-like activity has been recorded in the root tips of plants (Short & Torrey 1972) and it is generally accepted that cytokinins are synthesized in the root tips and then transported via the xylem to the above-ground plant organs (Short & Torrey 1972; Letham & Palni 1983). As the meristem of the root tip has been shown to be the site of cytokinin synthesis, numerous experiments involving the surgical removal of the root tip have been performed. These have yielded contradictory results (Torrey 1956; Pecket 1957; Böttger 1974; Wightman & Thimann 1980; Biddington & Dearman 1982/1983). Of significance is that the presence or absence of the root tip influences the formation of lateral roots. As a result, a role for cytokinins in root formation was postulated. The presence of cytokinins in root tips, or their application to them, inhibited root formation (Scott 1972; Torrey 1976). A gradient of cytokinins occur along the root axis (Torrey 1962) and Wightman *et al.* (1980) reported that, in pea seedlings, different levels of free cytokinins could be extracted from 5 mm above (9.7×10^{-5} M), and 20–25 mm above the root apex (8×10^{-5} M).

The nature of the cytokinin present in, or applied to, the root tip also influences lateral root formation and emergence (Wightman *et al.* 1980). Synthetic cytokinins are less inhibitory in lateral root formation than natural cytokinins (Forsyth & van Staden 1981) and free base cytokinins are more inhibitory than their ribosides (Wightman *et al.* 1980; Stenlid 1982).

Experiments using benzyladenine as a substitute for the root tip showed that this substance was inhibitory (Hinchee & Rost 1986). The cotyledons appeared to produce a substance which either counteracted or diluted the cytokinin effect, and removal of them increased sensitivity to the applied cytokinin. The results are also influenced by the time of application (Eriksen 1974) and the concentration of cytokinin used. Lateral root initiation is inhibited by concentrations of 10^{-2} to 10^{-5} M cytokinin (Wightman *et al.* 1980; Biddington & Dearman 1982/1883; Hinchee & Rost 1986). At concentrations between 10^{-6} and 10^{-8} M, promotive effects were observed (Eriksen 1974; Wightman *et al.* 1980; Biddington & Dearman 1982/1983). In culture, optimal lateral root emergence occurred at concentrations of 10^{-8} M (Finnie & van Staden 1985). These results should be viewed with caution as they could be affected by the amount of cytokinin assimilated by the plant organ, the rate of transportation, its stability and thus the degree of metabolism, and finally the receptivity of the tissues to which the cytokinin is applied. Bearing these problems in mind, our experiment was designed to establish (i) the interaction between the shoot (auxin) and the root (cytokinin) using surgical treatments, and (ii) the concentration at which the cytokinins,

zeatin and benzyladenine are either inhibitory or promotory, with respect to lateral root development and emergence.

Materials and Methods

Seeds of *Pisum sativum* L. cv. Greenfeast were immersed in running tap water for 15 h, sterilized in 0.5% NaOCl, rinsed and incubated at 25°C between filter paper for 48 h. When necessary the number of lateral root primordia (LRP) present was determined microscopically by staining in aceto-carmine and then clearing the roots with 60% lactic acid. For statistical purposes, 50 seedlings were used for each experiment and results analyzed by multifactor ANOVA.

For all experiments, seedlings with a radicle length of 30 mm were selected. Root tip removal was achieved by decapitating 3 mm of the root tip and the results were recorded 0, 12, 24, 36, 48, 60 and 72 h later. In experiments involving the shoot tip, it was removed either alone or in combination with the root tip. The cytokinins zeatin and benzyladenine were made up at concentrations of 10^{-4} –10^{-9} M and the solutions solidified with 1% agar in Elisa plates. Pea seedlings were then surgically treated (removal of root tip, shoot tip, or both) and the radicle or radicle stump was inserted into the agar. Results for all experiments were recorded 36 h after treatment. For all experiments primary root (radicle) length, number of lateral root primordia (LRP), number of emerged lateral roots (ELR), length of emerged lateral roots, and length of the lateral root free zone (LFZ) were determined.

Results

Roots of intact seedlings increased linearly with time (Figure 1) but root tip decapitation stopped elongation. In control seedlings, the number of LRP increased over the first 36 h (Figure 2). Removal of the root tip did not change this pattern of LRP formation, and the number of LRP formed increased significantly up to 48 h. There was no increase observed after 60 and 72 h.

Lateral root primordia were produced at a specific distance behind the root meristem. The zone where no roots are produced is known as the lateral root free zone (LFZ). The length of this zone did not change much with time if the root meristem was left intact (Figure 3). However, removal of the root apex reduced the length of the LFZ. Within 24 h this reduction was three-fold (Figure 3) and by 72 h no LFZ could be observed (Figure 3).

The emergence of lateral roots in intact roots began after 24 h.

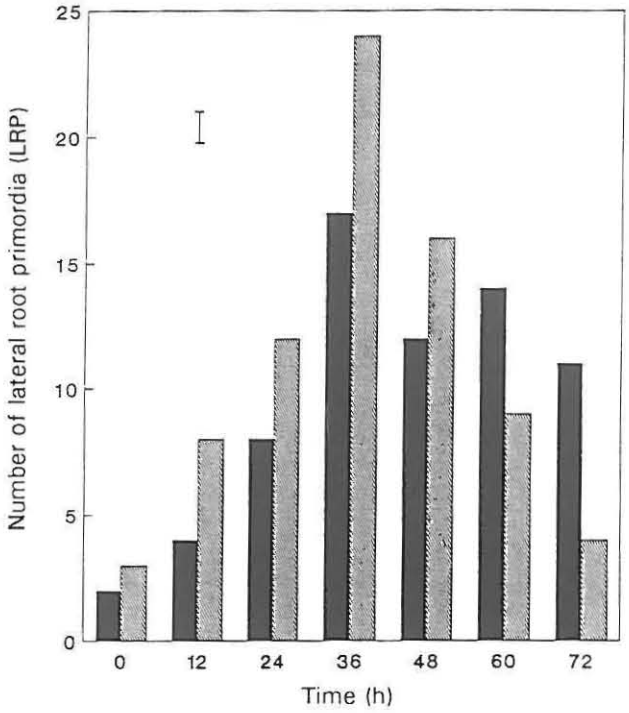

Figure 2 The effect of root decapitation on the initiation of lateral root primordia. Root left intact (■); first 3 mm of tip removed (▨). Only non-emerged root initials were counted. Bar represents the 95% confidence limit.

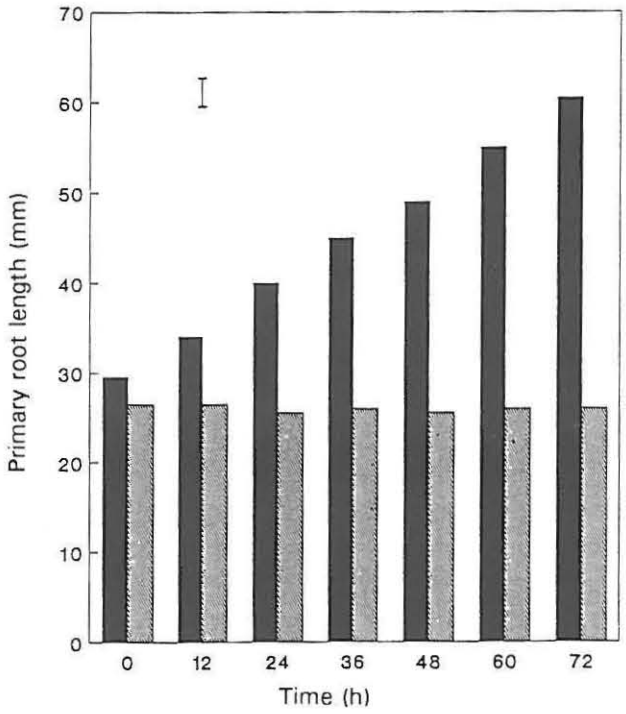

Figure 1 The effect of root tip removal on the primary root length of pea seedlings, with time. Root left intact (■); first 3 mm of tip removed (▨). Bar represents the 95% confidence limit.

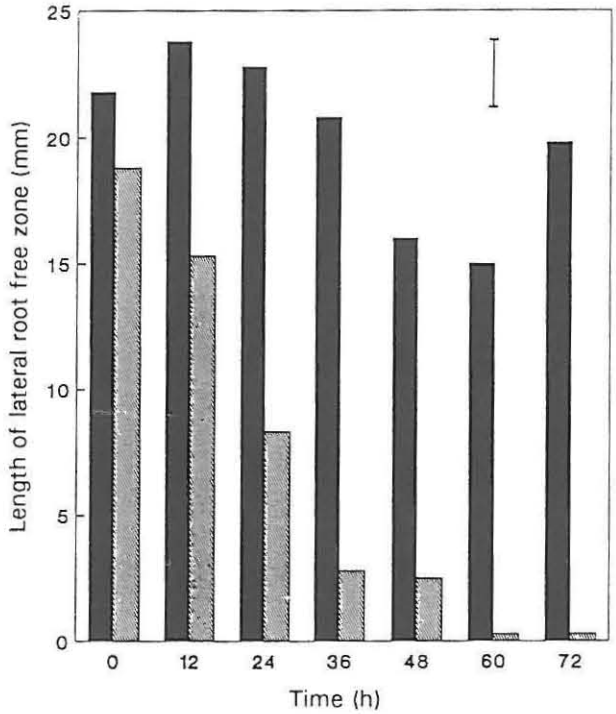

Figure 3 The effect of root tip decapitation on the length of the lateral root free zone (LFZ). Root left intact (■); first 3 mm of tip removed (▨). Bar represents the 95% confidence limit.

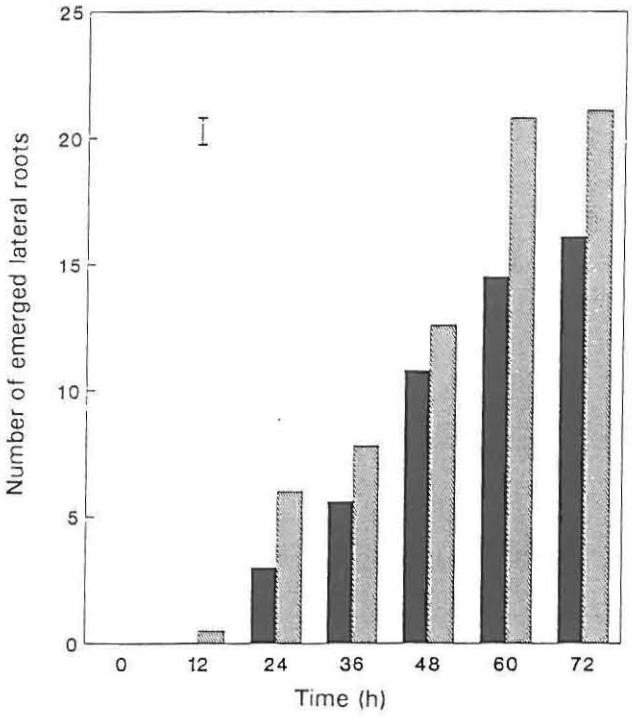

Figure 4 The effect of root decapitation on the number of emerged lateral roots (ELR) in pea seedlings. Root tip intact (■); first 3 mm of tip removed (▨). Bar represent the 95% confidence limit.

With removal of the root tip this process was accelerated to 12 h (Figure 4) and the number of roots that emerged increased with time (Figure 4), with maximum emergence occurring within 60 h (Figure 4). Following root tip removal, the length of emerged lateral roots (ELR) increased with time and was greater than in the controls (Figure 5).

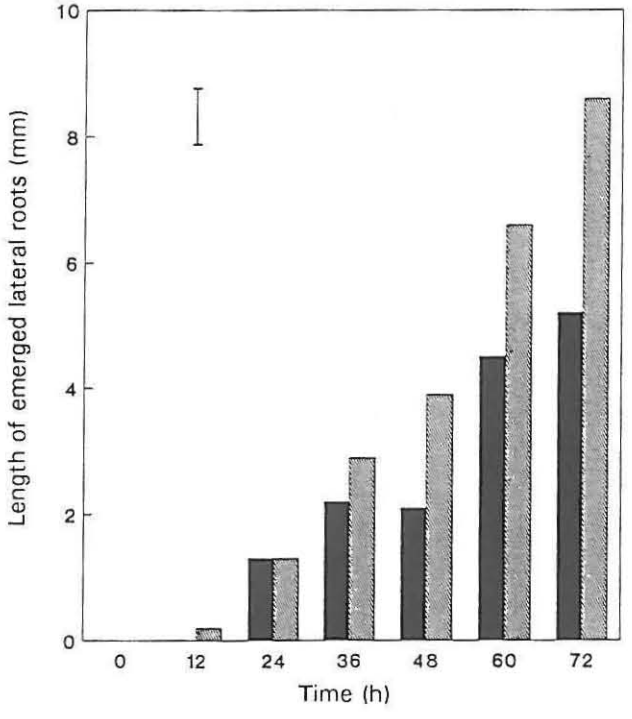

Figure 5 The effect of root decapitation on the length of the emerged lateral roots (ELR) in pea seedlings. Root tip intact (■); first 3 mm of tip removed (▨). Bar represents the 95% confidence limit.

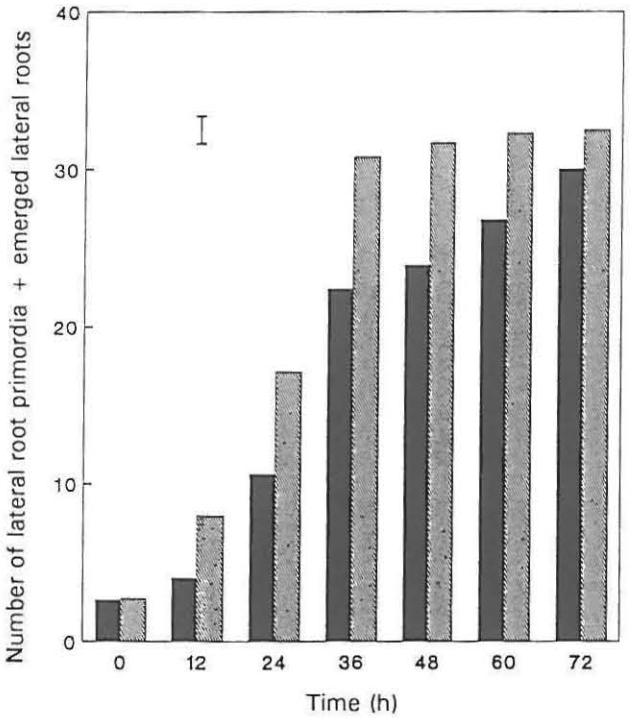

Figure 6 The effect of root decapitation on the rooting potential of pea seedlings. This parameter was obtained by adding the total number of lateral root primordia recorded and the number of emerged lateral roots. Root tip intact (■); first 3 mm of tip removed (▨). Bar represents 95% confidence limit.

Establishing the rooting potential of seedlings by combining the total number of LRP plus the emerged lateral roots, it is clear that the process of root initiation, formation and emergence was complete within 36 h after decapitation (Figure 6). The rooting potential of control seedlings was significantly lower at this time, however, potential increased steadily and by 72 h reached the same value as that of decapitated seedlings (Figure 6).

A comparison of percentage of LRP to ELR revealed that the process of emergence started earlier once decapitation had been effected. In intact seedlings, only 50% of primordia emerged within 72 h, whereas there was an 80% emergence in decapitated seedlings (Table 1).

Length of the primary root was not affected by removal of the shoot tip, but removal of the root or the root and shoot tips inhibited growth of the primary root (Table 2). In the case of lateral root primordia (LRP), removal of the root tip resulted in a greater number of primordia being formed when compared with controls. Shoot tip removal significantly reduced the number of primordia formed (Table 3). Interestingly, simultaneous removal of

Table 1 A comparison of the percentage of root primordia and lateral roots that had emerged over a period of 72 h in control and root-decapitated (3 mm) pea seedlings

		Time after decapitation (h)						
Treatments	Parameter	0	12	24	36	48	60	72
Control	% primordia	100	100	74	76	53	49	50
	% emergence	0	0	26	24	47	51	50
Decapitated	% primordia	100	92	66	76	58	24	20
	% emergence	0	8	34	24	45	76	80

Table 2 The effect of root tip, shoot tip, or root and shoot tip decapitation on the length (mm ± S.E.) of the primary root of pea seedlings. All treatments were compared with the intact seedlings. Root length was measured at 0 and 36 h

	Time after treatments (h)	
Part decapitated	0	36
Control	30.3 ± 0.4	42.5 ± 0.8
– Root tip	28.5 ± 0.3	28.8 ± 0.3
– Shoot tip	30.3 ± 0.3	39.5 ± 0.5
– Root and shoot tips	28.2 ± 0.2	28.6 ± 0.3

Table 4 The effect of root tip, shoot tip, and root and shoot tip decapitation on the length (mm) of the lateral root free zone (LFZ) (± S.E.) of pea seedlings. The LFZ represents the distance from the root tip to the most proximal lateral root primordium

	Time after treatment (h)	
Part decapitated	0	36
Intact control	21.2 ± 0.8	16.2 ± 0.9
– Root tip	17.6 ± 0.6	2.4 ± 0.6
– Shoot tip	18.1 ± 0.8	21.2 ± 1.2
– Root and shoot tips	16.9 ± 0.5	6.9 ± 1.4

both root and shoot tips had less of an inhibitory effect on root primordia formation than when the shoot tip alone was removed (Table 3). Root tip removal seemingly overrides the adverse effect of shoot tip removal. Length of the lateral root free zone (LFZ) was affected by various decapitation treatments, and root tip removal resulted in a decrease in the LFZ (Table 4). When only the shoot tip was removed the LFZ was much longer than in the controls.

Root and shoot tip removal severely reduced the LFZ but to a lesser extent than where only the root tip was removed, again showing the significant influence of the root tip on the system. On root tip removal, the number of emerged lateral roots (ELR) increased two-fold (Table 5). No roots emerged when the shoot tip or both the root and the shoot tips were decapitated.

A combination of applied zeatin or benzyladenine (BA) and decapitation treatments had little effect on the length of the roots of pea seedlings (results not shown). However, both zeatin and BA at concentrations of 10^{-8} and 10^{-9} M significantly increased the length of intact roots after 36 h. No root elongation occurred when the cytokinins were applied to seedlings of which the root tip or the shoot and root tips were removed. However, if only the shoot tip was removed, both cytokinins stimulated root elongation (10^{-8} and 10^{-9} M), albeit to a lesser extent than with intact seedlings.

As indicated earlier, root tip removal normally results in the formation of more LRP. In both intact and root tip-decapitated seedlings, the application of zeatin and BA at 10^{-4} to 10^{-6} M inhibited lateral root formation (Figure 7). Removal of the shoot tip had the greatest inhibitory effect.

Higher concentrations of zeatin or BA applied to the roots of intact or decapitated plants increased the length of the LFZ (Figure 8). However, there was a pronounced reduction of this zone whenever the root tips were removed.

In treatments where the shoot tip and the shoot plus root tips were removed, no lateral roots emerged, irrespective of the concentration of zeatin applied. In the intact roots, 10^{-4} to 10^{-6} M zeatin inhibited lateral root emergence (Table 6). At concentrations of 10^{-7} to 10^{-9} M, this was stimulated in both intact and root tip-decapitated plants.

Discussion

Lateral root production occurs in three phases, root primordia initiation, the development of these primordia into roots and finally, the emergence of these roots through the epidermis. It is possible that each phase is controlled by a different set of conditions. Root tips are thought to produce compounds that are inhibitory to lateral root formation (Short & Torrey 1972; Martin & Wong 1985) therefore, removal of the root tip promotes lateral root development (Torrey 1956; Böttger 1974). In the present study and that of Wightman & Thimann (1980) this trend was transitory. Root tip removal resulted in formation of root primordia 12 h earlier than in intact roots. Emergence of lateral roots was also accelerated by 12 h. This time difference resulted in lateral roots being more and longer than in the controls. The root tip greatly reduced the number of lateral roots formed over the 72-h experimental period. It appears that the root tip plays a major role in the last phase of lateral root development – emergence, partly as a result of nutrient diversion towards newly formed root initials (Wightman & Thimann 1980). Removal of the root tip also changed the site (position) of lateral root emergence, which then emerged in the former LFZ. This may be the result of either acropetally or basipetally moving promoters or due to the elimination of acropetally moving inhibitors. Cytokinins are thought to act as

Table 3 The effect of decapitation on the number of lateral root primordia (± S.E.) initiated after the decapitation of the root tip, shoot tip, or root and shoot tips

	Time after treatment (h)	
Part decapitated	0	36
Intact control	3.3 ± 0.4	11.6 ± 0.8
– Root tip	3.2 ± 0.3	14.3 ± 1.2
– Shoot tip	2.9 ± 0.3	3.0 ± 0.5
– Root and shoot tips	2.8 ± 0.2	5.3 ± 0.9

Table 5 The effect of root tip, shoot tip, or root and shoot tip decapitation on the emergence of lateral roots in pea seedlings after 36 h. Results are expressed as the mean ± S.E.

Part decapitated	Number of emerged lateral roots (ELR)
Intact control	2.4 ± 0.7
– Root tip	5.4 ± 0.9
– Shoot tip	0
– Root and shoot tips	0

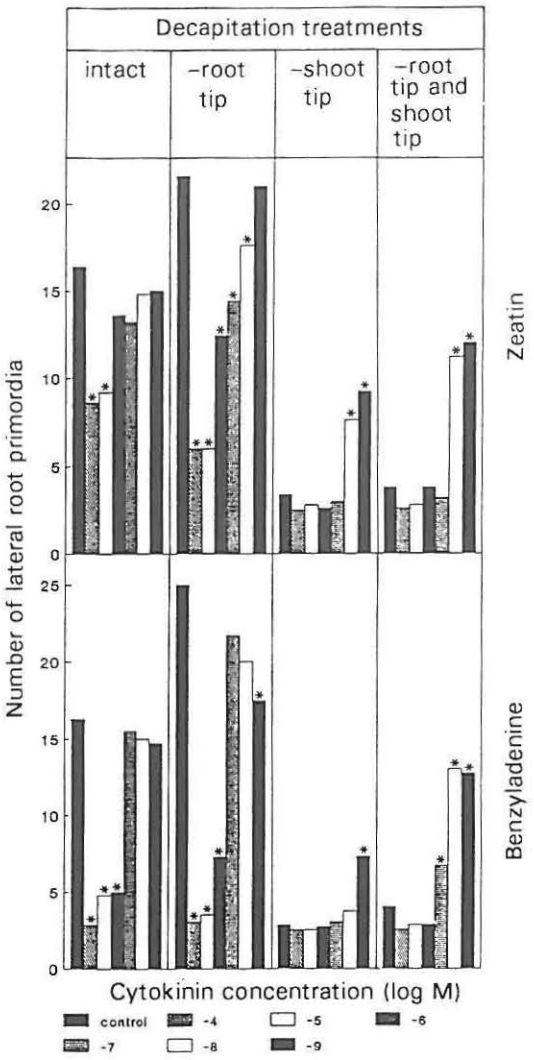

Figure 7 The effect of root-applied zeatin and BA in combination with different decapitation treatments on lateral root primordia formation in pea seedlings 36 h after decapitation. Asterisks indicate values significantly different from the controls.

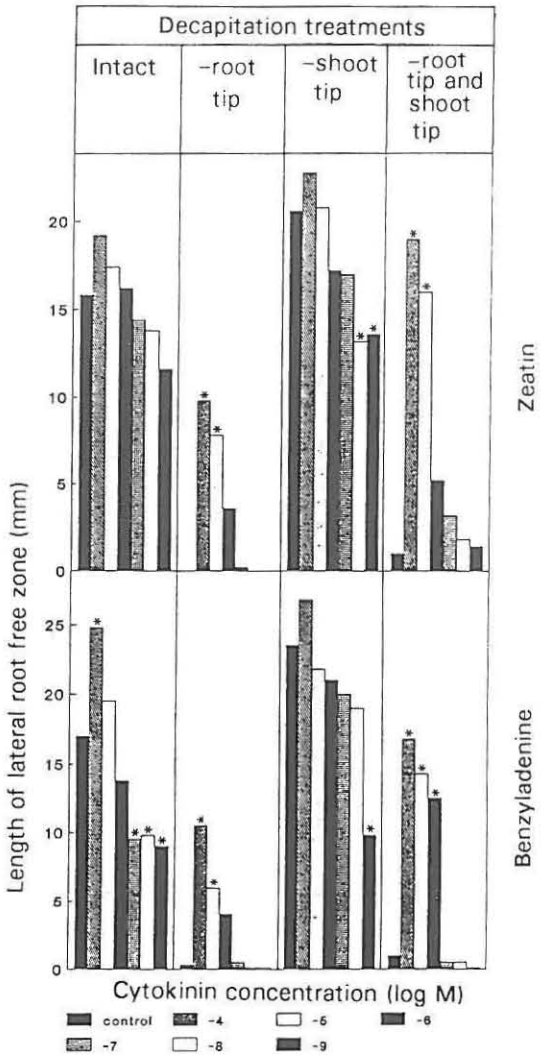

Figure 8 The effect of root-applied zeatin and BA in combination with different decapitation treatments on the length of the lateral root free zone in pea seedlings 36 h after decapitation. Asterisks indicate values significantly different from the controls.

such inhibitors and to be involved in the regulation of auxin (promoter) levels by activating IAA oxidase and thus reducing endogenous levels of auxin (Bourquin & Pilet 1990). A wounding response could also be involved, as ethylene in combination with auxin is known to stimulate rooting (Mandal & Basu 1982).

Removal of shoot tips completely inhibited lateral root emergence, yet lateral root primordia were still initiated, albeit in reduced numbers. Geissbuhler (1953) reported that cotyledon removal suppressed lateral root development. It therefore appears that the shoot and cotyledons provide factors directly involved in lateral root emergence.

This reduction in root primordia and inhibition of lateral root emergence agrees with the findings of Wightman & Thimann (1980) but not with those of Hinchee & Rost (1986), who

Table 6 The effect of root-applied zeatin on the number of emerged lateral roots in pea explants after the decapitation of the root tip, shoot tip, or root and shoot tips after 36 h. Results are expressed as the mean ± S.E.

Part decapitated	Zeatin concentration (M)						
	0	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
Control	2.2 ± 0.5	0	0	1.0 ± 1.0	3.4 ± 0.8	8.0 ± 3.6	8.0 ± 1.56
– root tip	5.8 ± 0.6	0	0	4.0 ± 0.9	11.2 ± 1.4	12.2 ± 2.9	10.2 ± 1.3
– shoot tip	0	0	0	0	0	0	0
– root & shoot tips	0	0	0	0	0	0	0

reported that root emergence does occur under these conditions.

Cytokinins, which are formed in the root tip and are transported via the transpiration stream (van Staden & Davey 1979), are widely considered to be the compounds (inhibitors) responsible for root apical dominance as they are present in the root as an upwards decreasing gradient (Short & Torrey 1972; Wightman *et al.* 1980). The present results obtained with zeatin and benzyladenine support this hypothesis. Zeatin and benzyladenine substituted for the root tip and inhibited lateral root formation. Both root primordium initiation and lateral root emergence were inhibited. Lower concentrations of cytokinins were less inhibitory. It has been suggested that high concentrations of zeatin may activate IAA oxidase, leading to a reduction in IAA levels and therefore less root growth. There is considerable evidence that cytokinins are responsible for the inhibition of lateral root formation and may determine the site where lateral root initiation will occur. Root emergence, and hence elongation of the newly formed initials, was enhanced in our study by cytokinin application. It therefore appears that the different phases of root growth are affected differently by cytokinins. That auxins may also be involved in the initiation of root primordia, root emergence and their site of initiation on the root is indicated by the combination of root and shoot tip decapitation experiments and those where cytokinins were applied. The regulator(s) produced by the shoot tip or cotyledons may play significant roles in the histological organization (position and hence the length of the lateral root free zone) and the emergence of the roots. The root initiation stage is apparently regulated by root tip-produced compounds.

Acknowledgements

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